DOI:
10.1016/j.fsigen.2018.11.019

Document Version
Peer reviewed version

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PII: S1872-4973(18)30274-6
DOI: https://doi.org/10.1016/j.fsigen.2018.11.019
Reference: FSIGEN 2011

To appear in: *Forensic Science International: Genetics*

Received date: 11 May 2018
Revised date: 14 November 2018
Accepted date: 26 November 2018

Please cite this article as: Burrill J, Daniel B, Frascione N, A Review of Trace "Touch DNA" Deposits: Variability Factors and an Exploration of Cellular Composition, *Forensic Science International: Genetics* (2018), https://doi.org/10.1016/j.fsigen.2018.11.019

A Review of Trace "Touch DNA" Deposits: Variability Factors and an Exploration of Cellular Composition

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Declaration of interest: none.

This work was supported by the Fulbright-King's College London Partnership Award.

Highlights:

- Amount of DNA deposited on touched items is highly variable and difficult to predict
- Ongoing research shows wide range of factors in variability of touch DNA transfer
- Little known about cellular/non-cellular components and DNA origin in touch deposits
- Anucleate corneocytes, free nuclei, nucleated cells, and cell-free DNA, may all be sources

Abstract

The use in courtrooms of forensic DNA typing results from presumably touched or handled items is increasing as the sensitivity of detection techniques improves. Research investigating how much DNA can be recovered from handled items, whether trace DNA can be detected under certain scenarios including varying degrees of indirect transfer, and factors which may influence these results is summarized here. Fundamentally, our current understanding of the cellular content of touch deposits and the origins of the potential trace DNA therein is extremely limited. Possible origins include anucleate corneocytes, fragmentary cells/nuclei, nucleated epithelial cells from hands, transferred nucleated cells, and cell-free DNA. Here we review the existing evidence for each possible source and consider remaining knowledge gaps regarding forensically relevant touch depositions.

Contents

1. Introduction

Recovering DNA left at a scene and matching it to someone who may have been responsible for a crime is now a ubiquitous tool in forensics. Improvements in scientific techniques have allowed better recovery rates and testing of not only body fluids, but DNA deposited through handling items [1]. The methods of recovery, extraction, amplification, and detection continue to be optimized and results are being generated from extremely limited amounts of DNA, often from multiple individuals [2]. Low template, multi-contributor results present analytical challenges and their interpretation is the subject of ongoing scientific and legal debate [3]. However, these results continue to be used in casework laboratories and courtrooms because of the perceived value their analysis provides to investigators about item handling or similar contact.

Biological samples with low levels of DNA not attributed to a specific body fluid are often referred to as “trace DNA” or, alternately, “touch DNA” as touching is assumed to be how they were deposited, yet their precise nature and origin are poorly understood. Published research about touch DNA typically answers one of two questions: 1) Which technique is best for this sample type? [4, 5, 6] or 2) How much DNA is detected after handling/recovery scenario X? [7, 8, 9] Only occasionally thus far has light been shed on a third, more fundamental, question: Where is deposited “touch DNA” coming from? [10, 11, 12]

The first question is of particular interest to practicing forensic laboratories, and numerous studies exist comparing recovery rates of trace DNA by collection method, usually discussing the yields from different swabbing parameters (e.g. technique [13], buffer [14, 15], material [16], etc.),

tape-lifting materials [17, 18], vacuum tools [19, 20], and electrostatic equipment [21], and even direct amplification [22, 23]. Efficiency comparisons of various DNA-affinity bead- and filter-based extraction methods also abound [24, 25]. Recovery, through-put and costs vary, as does suitability for specific substrates and sample volumes [26, 27, 28]. A more in depth survey of developments for the collection, extraction, quantification, amplification, detection and interpretation of low-level samples has been furnished elsewhere by van Oorschot and colleagues [2] and is not the focus of this review.

The second question, that of how much DNA can be detected following specified scenarios, is of greater interest to investigative or legal teams attempting to answer case-specific inquiries about the likelihood of DNA recovery under an alleged set of circumstances. Current research efforts to quantify DNA transfer and isolate the factors influencing its variability are summarized here.

The third question concerns the biological or cellular origins of the DNA recovered from touched objects and remains largely unanswered [10]. The fundamental question of precisely where "touch DNA" is coming from has significant implications for interpretation of results in forensic casework, testimony and research. Resolution and characterization of the cellular and subcellular components of touch deposits will inform how scientists recover, analyse and interpret this evidence in the future.

2. Trace DNA – a concept and a process

"Trace DNA" as nomenclature may refer to DNA from any source present in low levels, usually recovered speculatively without visual detection of a body fluid or stain. This could include DNA left by speaking or sweating onto a surface, or "wearer DNA" recovered from the fabric of clothing; it would also include "touch deposits," which we will define as material left by the touching or handling of a substrate. No precise amount or tissue of origin or method of analysis is included in the definition of "touch DNA" as the DNA recovered from these deposits. This in turn would include "transfer DNA" which may not have originated on an individual's hands but was subsequently deposited by them. Definitions such as this can vary between research publications and should be considered as the field moves forward.

Early research into detectable human DNA on objects from contact other than with blood, semen or saliva was concurrent with the widespread development of forensic DNA testing generally [29], and included several reports listing recovered amounts of DNA from handled objects [30], as well as consideration of how DNA may transfer between individuals and items [31, 32].

2.1. Amounts of trace DNA

Studies reporting the amount of DNA deposited by touching/wearing/handling items have demonstrated great variation between and within individuals, making predictions about "expected" amounts of DNA on these items generally impractical [33]. A summary of DNA levels recovered from touched surfaces is included in Table 1. Although overall trends such as worn clothing, especially headgear, retaining more DNA than handled items are often reported [34], the inherent variation in DNA deposition and recovery is clear, ranging from 0 ng to nearly 170 ng of DNA measured (see Table 1). The individual studies themselves also vary greatly in their deposition and quantification methodologies, rendering direct comparisons challenging and predictive conclusions unreliable.

The idea of "good shedders" and "bad shedders" (i.e. people who generally leave more or less DNA upon contact) appeared at length in the literature in 2002 [39, 40], although the disparity between individuals had been reported previously [32]. While some have found this categorization overly simplistic due to the wide intra-individual variations seen [7], the categorical terminology has

continued in research and testimony [41, 42, 43]. Some authors have reported increased DNA "shedding" or "sloughing" in men versus women [44], and in young males compared to older ones (the same trend was not observed in females), but significantly, over ¾ of subjects changed status (good/ intermediate/ poor) between trials three days apart [45]. Others concluded that while children are typically "better" shedders and the elderly somewhat "worse," adults in the categories in between are significantly less predictable [46]. Although high intra- and inter-individual variation is consistently reported [47, 48], many authors report that those subjects whose handled items yield either more or better quality DNA are a quantifiable group [44, 47, 49, 50]. No clear biological basis for this trend has been identified and initial amount of shed DNA could impact subsequent comparison of other variables influencing DNA transfer. It remains unclear what precisely is being "shed" to deposit DNA and where, amongst the constituent parts of a touch deposit, detectable DNA originates. Knowledge of what the deposited material actually is may allow researchers to explain the shedding variation between people, predict DNA deposition levels and recover more DNA from these sample types in the future.

2.2. Persistence and interactions of trace DNA in its environment

Once DNA has been deposited, the question arises of how long it will remain on a surface to be later collected and tested, and what factors effect that persistence. Longer time intervals between original deposition and transfer/recovery seem to lower DNA yield, but mostly in conjunction with the environmental conditions [51]. Storage in a laboratory environment resulted in no significant drop in DNA recovery levels from either "wearer DNA" on underpants after 12 weeks [52] or DNA slides after 6 weeks [51]. Exposure to potentially degrading environments (e.g. a sunlit windowsill) has been shown to lower this persistence significantly, with recovered DNA displaying clear degradation in typing results after 2 weeks [51]. Total DNA recovered from knives immediately after use compared to a week later, was reported to be significantly higher in only 50% of subjects [53]. There is limited systematic investigation of the time course of DNA degradation, particularly in either casework-like touch deposit samples or over significant amounts of time.

In addition to environmental factors, DNA persistence on items is affected by subsequent contact with other items or people. DNA collected from individuals touching a shared keyboard for example was seen only up to 8 days after deposition [54], demonstrating a removal of DNA during later interactions. There is interest in whether the timing or order of individuals handling an object can be inferred from the touch DNA evidence; for example, it was previously believed that the major contributor will be the person who handled the item most recently as their DNA would displace or overwhelm prior depositions [1]. While this holds a logical appeal and overall this pattern can be observed in the data on the subject [45, 55], exceptions arise and broad conclusions about activity or handling order could be erroneous [56] as there is wide variability. There are instances of the most recent handler's DNA or even the DNA of the depositing hand being absent or only present as a partial minor contributor [57, 47]. Some of this variability could be clarified by future characterization of the DNA within touch deposits, as its tendency to transfer at contact may be a function of where it is located within or relative to a cellular structure and whether it has been previously fragmented.

DNA persisting on substrates from previous contact can critically affect how much and whose DNA is recovered now that techniques are sensitive enough to detect this retained background DNA [58, 59, 60]. Prior to contact of interest in an investigation, human DNA from an unconnected person could be present on items, derived from previous handling, secondary or subsequent transfer, owner/manufacturer contact or even contactless proximity [61]. Levels of detectable foreign background DNA have been documented on a large range of samples from

everyday objects [62] and environments [63, 64], including clothing [52, 65], computer equipment [54], and human bodies [66, 67], particularly fingernails [68, 69]. Perhaps most concerning is the persistence of background DNA in forensic laboratories [70, 71], on scientific equipment [72, 73], in autopsy suites [66, 74], in police stations [54], and on manufactured consumables [75, 76], including gloves [77], all of which are likely to come into contact with evidence subsequently tested for DNA with very sensitive methods. It is important that the content of these residual DNA deposits is understood; this may allow us either to prevent their interference with casework through selective collection or to place their detection on evidence in its appropriate context should persistent background DNA prove to be omnipresent.

2.3. Trace DNA as a clue about timing or activity

Having recovered a touch DNA profile from a piece of evidence in a case, it is of obvious interest to evaluate if conclusions can be made about whether that individual possessed that item or when and in what way they interacted with it (e.g. did they fire the gun or just shake hands with someone who did?). Studies into this have again produced varied results, as factors such as length of contact, type of contact, amount of DNA shed, and extent of previous contacts before interaction with the item of interest may all play a role.

Levels of DNA ranging roughly from 0 ng to 75 ng have been reported from items considered to be in regular use by an individual [33, 78]. These items include handles, keys, pens, etc. [30, 32], as well as sheets and clothing presumably in prolonged contact with people's bodies [42, 52]. Recovered DNA from worn clothing may often consist of mixtures [79], and can include that of the wearer as well as an individual touching the items, as well as unattributable background DNA, leaving ambiguity as to what interaction gave rise to the DNA deposition [52]. Trends in published DNA data have suggested "common" or "likely" outcomes from a specific interaction such as wearing a shirt with a collar [80], but exceptions are usually observed. The numerous variables confound predictions, as both DNA levels and profile quality lack the consistency required for broad ex post facto inferences.

One category of studies investigate DNA on items following specific controlled actions, such as knife handling or hand-washing [56, 81]; increased pressure of finger contact, for example, has been shown to result in higher levels of DNA recovery [82]. A second genre of studies analyse a series of more "realistic" scenarios with less control but potentially more immediate relevance to casework [51, 83, 84, 85]. Both groups yield a wide range of typing results in terms of quantity, quality and complexity (see Table 2). Handling of more items in advance of touching the tested item has been demonstrated to decrease deposited DNA material, thus low DNA recovery could indicate either limited contact or contact after previous handling of other items, with no distinctions in the resulting data [86, 87], or for that matter distinction from the data after contact of a low-shedder [50]. A summary of DNA detection results from various studies following regular, controlled or realistic use is presented in Table 2.

Some forensic practitioners do evaluate activity-level propositions through Bayesian network assessments or are working toward parameters for doing so [88, 89], although caution is still urged that conclusions about the source of DNA are not conflated with conclusions as to how it arrived there [90], and that more research is required to strengthen approaches to activity assessment [91, 92]. Although potentially useful to investigators or fact-finders should the scenario and protocol at issue in the instant case be identical, the overall variability in these types of studies make it currently problematic to render broad conclusions about expected previous activity or item use based on residual detected amounts of DNA or DNA profiles.

2.4. Transfer of trace DNA after initial deposition

Secondary and subsequent transfer refers to the mechanism by which DNA can be deposited on an item not through contact with the biological source of the DNA but with an intermediate person or item. Despite earlier doubt about the impact of secondary transfer [32], this occurrence has been repeatedly demonstrated and is now widely accepted [48, 50, 93]. Particularly as testing sensitivity grows, secondary, tertiary and subsequent transfer events produce detectable DNA profiles on items never handled by the DNA contributor; this detection can follow innocuous interaction such as handshakes [94] or co-laundering of clothing [65, 95], or even routine movement of people and equipment around a forensic DNA laboratory and office [71].

However, there is little consistency in when and how it will occur, how much DNA may be transferred, or how many interaction events such transfer will persist through [54, 56, 96]. Foreign, or non-self, DNA transfer has been reported as “minimal” on the majority (85%) of touched samples by some [45], while other research has found transferred DNA consistently, with levels exceeding primary DNA deposition in 20% of samples [94]. Still other researchers report the logical progression of foreign DNA acquired and redeposited on a knife handle decreasing in parallel with an increase in intermediate DNA-free handled items [57]. DNA has been demonstrably transferred by handling both with and without gloves [97], from a range of items to both secondary and tertiary objects [98]. The transferred DNA may yield profiles ranging in quality from non-existent to complete and database-uploadable [97, 98], following either prolonged or brief social contact [99], leaving unpredictability (see Table 2).

2.5. Substrate or surface interaction with trace DNA

One important factor in the variability of trace DNA is the surface from and onto which the biological material is deposited. Proof-of-concept studies report the deposition of DNA to greater or lesser degrees on surfaces that collect DNA well or not. DNA has been recovered successfully from a wide range of substrates including metal cables [6], lipstick [101], car interiors [102], shoes [42, 103], firearms and ammunition [104, 43], plastic bags [97, 105], sheets [78], paper [106, 107], and of course, fingerprints [30, 108]. Generally rough and porous surface are reported to be superior collectors of DNA to smooth ones; wood is preferable to fabrics followed by glass [8], cotton being better than plastic upon consistent handling [48].

Transfer as well as deposition can be significantly influenced by surface. If the DNA in question is to transfer from a primary item to a secondary one, the tendency to release biological material may improve with the substrate being less porous, such as plastic or glass [4], while tendency to collect DNA as the secondary substrate may increase with porosity [109]. This is also apparent from results that gloved thumbs transferred more saliva than uncovered ones [110]. Moisture in the sample or surface also improves transfer of DNA either from the body or an intermediate surface [48], as does introducing friction into the contact [48, 65]. This is perhaps consistent with reports of transferred DNA being recoverable from clean clothing items co-laundered with used sheets or stained fabrics, as moisture, friction and continuous exposure would all be expected [65, 111].

Along with shedding propensity, activities, timing, surface, friction and similar factors discussed above, partial explanation for the variability in DNA deposition, persistence, and transfer may be that people simply touch a large number of items as well as themselves surprisingly frequently (roughly 15 contacts per minute) [112] and that each such contact has the potential to add or remove detectable DNA. Each of those contacts is subject to all the factors mentioned, leading to ubiquitous DNA transfer events with almost endless variability.

Studies investigating this second question, that of DNA recovery under certain deposition or transfer scenarios, have demonstrated such wide variability that conclusory statements inevitably advise caution in interpretation of these results in casework. Reliable statements of the events, time or nature of physical contact based solely on DNA typing results are inadvisable, meaning that predictability of touch DNA deposits still eludes us. What might inform our understanding of that variability further is an answer to the third question about the actual content of these DNA-containing touch deposits, which is still imprecisely understood [113]. The origins of DNA within a touch deposit have only begun to be directly queried [49, 114, 115].

3. Composition of Touch Deposits

Historically, scientists assumed DNA deposited by touch came from sloughed off outermost skin cells [31], and that narrative persists in courtroom testimony and publications today [41, 42, 49, 81, 97], despite limited evidence to support it. Data in studies of touch deposits is typically in the form of DNA typing results rather than any microscopy or cellular characterization, thus demonstrating empirically only the presence of DNA and not its provenance.

We propose that DNA may originate in one of several places: shed keratinocytes or their constituent parts from the outer layers of an individual's hands, nucleated epithelial cells from other fluids or body parts in contact with one's hands (e.g. saliva, nasal fluids, eyes), or from cell-free DNA reserves either endogenous to the hands (e.g. sweat) or transferred onto the hands from the aforementioned fluids (see Figure 1). As there has been little inquiry into these distinctions previously, what data there is for each of these potential sources must be gleaned from the literature on touch DNA, transfer, epithelia and cell-free DNA generally. Herein we discuss the current state of the evidence supporting anucleate or fragmentary keratinocytes from the hands, nucleated epithelia from hands or from other parts of the body, and DNA outside of a cellular structure as potential sources of what scientists have been researching at length as "touch DNA."

3.1. Cells originating in the hands

When biological material is deposited by hands onto a surface, it is logical to consider whether the hands themselves are generating it. Individuals discussed above as "good shedders" may be leaving higher levels of DNA deposition due to elevated populations of any of the putative sources in Figure 1, including "dead" skin cells, subcellular fragments or intact nucleated cells.

3.1.1. Anucleate corneocytes

The outermost layer of the epidermis, the *stratum corneum*, consists of 8-13µm thickness of flattened, fully differentiated keratinocytes (known as corneocytes, see Figure 2) that have undergone keratinization, losing their nuclei and organelles, cross-linking their keratin filaments, and reinforcing their cell membrane with hard proteins [116, 117]. As many as 1000 cells/cm^2/hr [118] may be shed by an adult human, in a process of active desquamation which occurs continuously [119], resulting in up to 10^9 corneocytes deposited from the body each day [120]. Some authors point out that these cells are corneocytes lacking nuclear DNA and thus should not constitute a significance source of touch DNA [11] even if recovered in large numbers. Therefore the common description of shed skin cells from hands as a putative source of touch DNA is in contrast to our basic biological understanding of the outermost layers of our hands and skin [62, 110].

Ambiguity remains as to whether these anucleate cells may retain detectable amounts of DNA. When touch deposits have been examined microscopically, typically no correlation has been observed between the number of anucleate keratinocytes and the amount of DNA recovered [114,

122], however this has recently been challenged by data from directly amplified DNA [49]. Both detectable [123] and negligible [122] levels of DNA from entire cell pellets of keratinocytes have been reported, leaving it unclear if these cells are a DNA source in touch samples. It has been reported that keratinocytes deposited by touching [124], as well as those in distal head hairs [125], presumed to be fully keratinized and inactive, have stained positive for DNA with multiple nucleic acid dyes and yielded detectable profiles. There is also data suggesting that corneocytes on hands may have removable surface-bound DNA [126].

3.1.2. Fragmented or degraded cells including free nuclei

Some authors suggest that the residual DNA in "stripped nuclei" or fragmented cell remains (resulting from the apoptotic or other cell death pathways accompanying keratinization) could be the major source of recoverable touch DNA and that "good shedders" may be those individuals with naturally higher rates of keratinocyte turnover [127]. This may be supported by published observations of more DNA deposited by individuals with drier hands [42] and with active skin diseases known to elevate keratinocyte turnover [128]. Nucleic acid staining of touched items and dead skin has shown clustered fluorescence distinct from a cellular structure which could be diffuse intracellular DNA [129]. Reported rabbit polyclonal anti-ssDNA antibody staining in the outermost skin tissue has revealed a few nuclei-like objects in the cornified layers of cells, suggesting either that these cells are not as entirely devoid of DNA as was previously thought or that compromised cells have left residual nuclei. The authors of this study (see Figure 3A) used skin samples from the neck rather than hands of 2 autopsy subjects, so applicability to hands of live individuals was unclear [130]. However, fingerprints from washed hands have been positively stained for DNA showing occasional free nuclei (see Figure 4B), suggesting endogenous fragments as a DNA source [131].

3.1.3. Nucleated epithelia

Since the outer layers of keratinocytes on hands lack nuclei, we must consider if "hand-endogenous" (i.e. not transferred from elsewhere on the body) nucleated cells may nonetheless be present on people's hands providing touch DNA. STR typing is routinely sensitive enough to detect 0.200 ng or less, (equivalent to approximately 30 nucleated cells [132]) and current methods detect profiles from almost single-cell levels (0.008-0.010 ng) [133, 134, 135]. Consequently, even a limited population of nucleated cells, possibly originating from eccrine sweat ducts and subsequently brought to the hands' surface, could be a contributing factor in touch deposit DNA.

Microscopic examinations have detected occasional nucleated cells (average of 2.66 and 5.66 for palms and fingerprint pairs respectively) from washed hands an hour after washing without interim contact (see Figure 4A), demonstrating this contribution in theory [131]; although where washing/contact parameters were not specified, nucleated cells may be transferred or endogenous and are typically many times outnumbered by the predominant anucleate corneocytes and cellular fragment populations [107]. Based on a small sample, DNA recovered was reported to be proportional to stripped nuclei but not to nucleated cells, suggesting the latter may not be making as much of a contribution as the former [127]. However, authors using a mouse anti-ssDNA IgM monoclonal antibody were unable to detect any DNA in the outermost epidermal layer of skin tissue from autopsy subjects (see Figure 3B), undermining the idea that either free or encapsulated nuclei were a part of skin surface deposits [115]. Studies examining nucleated versus anucleate cells do not always distinguish between washed and unwashed hands, nor do they consider cell-free sources in their DNA measurements, so more precise localization remains elusive, although endogenous nucleated cells remain a possibility.

Unfortunately, sample sizes, experimental design, and body locations limit conclusions to be drawn thus far about the potential for anucleate, fragmented or nucleated cells from the hands to yield the constituent DNA in touch deposits. It remains ambiguous whether corneocytes or their fragmentary parts may contain residual detectable DNA despite the absence of nuclei, or how nucleated cells may themselves arise in appreciable numbers from hands over time.

3.2. Cells originating exogenously to the hands

Nucleated epithelial cells are a rich source of DNA, so although they may not be arising in great numbers from the hands themselves, they could still constitute a significant source of touch DNA deposited if they are transferred onto the hands from elsewhere on the body. These nucleated cells may come from one's own body or from external contact (giving rise to non-self or foreign DNA profiles when tested). Of interesting note is the presence of diverse microbial DNA present on hands [136, 137, 138], both before and after washing [139, 140]. Although not the subject of this review, whenever DNA quantification methods which are not specific to human DNA are used, this must be considered as a possible source. Since the presence of non-self, human DNA (potentially from nucleated cells, as well as anucleate or cell-free sources should those prove to be contributory) accumulated extrinsically is so widely observed in touch deposits [56, 69, 141], it is logical that similar transfer would occur with one's own DNA and that some of the material on one's hands comes from elsewhere on one's own body. Although the implications for forensic casework of complex human mixture interpretation presented by foreign DNA accumulating in touch are beyond the scope of this review, mixture interpretation will be greatly informed by a better understanding the dynamics of individuals' own touch deposits.

A few studies have considered that cells accumulating on the hands from one's own face or body or clothes may be a source of handling-deposited DNA [49, 115]. Saliva is a realistic source of high levels of self-DNA [142], and has been demonstrated contributing to major DNA profiles after secondary and tertiary transfer between fingers, pens and surfaces [110]. Sebum has become another source of recent interest. Palms lack sebaceous glands, so sebum-derived DNA is of hand-exogenous origin. In one study, DNA in fingerprints of washed hands was undetectable, but after rubbing sebaceous skin of a second individual, the fingerprint DNA recovered was that of the second individual rather than of the donor. No DNA profile results were detected in the fingerprints after the washed hands had rubbed non-sebaceous skin of the second individual [115]. These results, together with the authors' observation of DNA staining in most cells present in sebaceous glands, but not in outer epidermal cells suggest sebum is a viable source of transferred self-DNA for touch deposits. The demonstration of the absence of self-DNA in fingerprints recovered immediately after washing, compared to the plentiful self-DNA recovered from fingerprints after contact with the donor's own skin suggests this as a clear possibility and raises the question of whether shedder status could be a result of varied sebum secretion levels.

The application of mRNA techniques to elucidating the anatomical source(s) of transferred DNA from hands has made important contributions. Body fluid identification by mRNA sequences is well researched [143, 144], but the identification of a forensic tissue source other than blood, saliva or semen is relatively recent [145, 146]. A wider range of markers including those for circulating and menstrual blood, seminal fluid, sweat, urine and saliva [147, 148, 149, 150, 151], as well as possibly nasal mucosa [62] and skin [113] have been identified. Specificity and sensitivity of markers vary, and the risk of overlap with related tissue types is of note. RNA extracts from skin swabs have been tested with "skin-specific" mRNA marker sets chosen based on literature searches and marker over-expression compared to other sources of epithelial cells such as vaginal fluid; markers include three

cornified envelope genes (LCE1C, LCE1D, LCE2D), an interleukin (IL1F7) and a chemokine (CCL27) [152], as well as corneodesmosin (CDSN), loricrin (LOR) and keratin 9 (KRT9) [145]. Some of these were subsequently multiplexed with other tissue markers for use on contact trace samples [153]. In a survey of samples from handled items, mRNA markers for skin were found in the majority; markers for specific non-skin tissues found in 15% of samples, which had significantly higher DNA yields and less degradation. This suggests that transferred biological material not originating in hands may contribute the highest quality DNA to touch deposits although not necessarily present in most deposits [154]. Although 85% of samples did not display a specific non-skin marker, it does not mean that 85% of touch deposits do not contain exogenous transferred material. The full range of fluids containing "skin" markers is unclear and they would of course be expected in skin beyond that of the hands, so any material acquired from touching one's own body surface cannot be distinguished from that originating in the hands. Therefore, we are unable to conclude specifically whether the detectable mRNA in touch deposits came from hands or elsewhere on the body.

The ability to parse out various cellular contributions to touch deposits by origins would clarify inquiry into where the DNA-bearing constituents of touch deposits originate. Distinctive visual (microscopic) traits of shed epithelia from vaginal, buccal and arm/head skin have been reported [155] and cytokeratin makeup may distinguish cells of mucosal or epidermal origins [156], but it is unclear yet how to completely resolve epithelia from saliva, nasal fluid, sweat, eye fluid, hands, etc. Work on tissue distinction using mRNA and immunohistology currently promises meaningful data concerning the potential for hands to acts as vectors for biological material from other parts of the body, particularly if the specificity can be improved to distinguish between epidermal locations.

3.3. Cell-free DNA

Cell-free DNA (cfDNA) is the most recent addition to the potential sources of touch DNA and the least thoroughly understood. Widely studied since its discovery in blood plasma in 1948, cfDNA is mostly of interest in cancer diagnostics and non-invasive prenatal screenings [157]. Its use in cancer research derives from the elevated concentration of cfDNA in the plasma of cancer patients, previously attributed to the high rate of apoptosis and necrosis associated with tumour growth [158]. Current debate about the origin and mechanism of release of cfDNA via apoptosis, necrosis or active release is ongoing. Elevated cfDNA levels are observed in patients with a range of conditions from cancer to inflammatory disease to trauma, and may be as high as 1000 ng/ml blood [159]. However, cfDNA is also present in healthy individuals' plasma, where reported levels range from 1.8-44 ng/ml [160] and may increase in response to non-disease states such as heavy exercise [161, 162].

Since many cell types actively release DNA to some degree [163], cfDNA beyond the bloodstream is not surprising. Its presence has been reported in sweat [12], saliva [164], semen [165], and urine [166]; cfDNA is also documented from touched items [167], sometimes even as the significant majority of the recoverable DNA [122]. It may transfer from individuals' hands to items via sweat, where levels of cfDNA have been shown to average 11.5ng/ml, but to vary by day within and between individuals [12]. When cell pellets and supernatants from real and simulated contact trace samples were extracted separately, very similar DNA typing results were found, suggesting an equivalency between cellular and cell-free DNA sources [167]. However, most studies quantifying or amplifying touch DNA do not separate these fractions, so results attributed to deposited cells may reflect cfDNA as well. Free DNA has reportedly been stained in fingerprints without nuclei, although the data was not published [130] and subsequent authors interpret this report as indicative of either ssDNA within a differentiated and flattened corneocyte or extracellularly located, thus leaving considerable ambiguity as to the qualities and origin of cfDNA in touch deposits [115, 131].

If the cfDNA in touch deposits originates from cfDNA populations present in the body (rather than becoming cell-free after deposition due to cell degradation), it will be important to characterize its fragmentation pattern and determine its cellular origin to fully understand the nature of the DNA reserves found on items. Touch DNA is widely reported to be degraded; observations of DNA freshly deposited from washed hands showing this pattern [131] is evidence that the DNA may have been fragmented in the body (such as cfDNA) rather than subsequently. Plasma cfDNA is notably double-stranded and heavily fragmented [168], with reports on the fragment sizes varying somewhat. The majority of results indicate fragments range from 150-200bp with smaller populations at multiples of those lengths, consistent with the DNA wrapping around a nucleosome one to five times during apoptosis [169]. However, various methods have reported typical cfDNA fragment lengths of 1200-1800bp, anywhere between 88-28,500bp, at 10bp increments 166bp and below [170], or mostly above 10,000bp, consistent with a necrosis mechanism rather than apoptosis [171].

Methods used to investigate fragment size distribution are not standardized for plasma cfDNA, ranging from electrophoresis, electron and atomic force microscopy to massively parallel sequencing and quantitative PCR of cancer markers [170]. These varied techniques have led to some of the conflicting fragment size results in plasma and fetal cfDNA, while the fragmentation in other sources of cfDNA has not been investigated at length. Quantitative PCR to estimate degradation based on short and long fragment ratios can inform researchers about the condition of touch cfDNA [172, 173, 174], although anything PCR-based risks overlooking fragments without primer binding sites, which is a concern if cfDNA in touch samples is fragmented similarly to that in plasma. When saliva samples were separated into their cellular and cell-free components, the latter required significantly higher input for reliable DNA typing results, possibly due to this fragmentation of the salivary cfDNA making successful amplification more challenging [164]. Research on cfDNA in any fluid faces the current challenge of lack of standardization in extraction and quantification methodology, particularly if overall quantity or quality rather than marker-specific detection is being queried (via PCR) [159]. Recent deep sequencing of plasma cfDNA has suggested that fragmentation patterns which indicate nucleosome positioning may be an indicator of cellular origin [175], which could be useful if extended to cfDNA in touch deposits. The lengths of fragments are important not only to biological understanding of this DNA source and its dynamics, but to determining if it is currently relevant to forensic testing applications or could be in the future.

4. Concluding Remarks

Research into the best processes for touch DNA recovery and interpretation continues and improvements will increase sensitivity and analytical methods in the future. Meanwhile, predictability of the exact mechanics of DNA deposition and transfer is still elusive, but more data is continuously being gathered on a wide range of handling scenarios and their tendencies to yield detectable DNA. Limited data is available on the cellular or acellular nature of the DNA in touch deposits, but further inquiry in this area could contribute to the search for predictability as well as our fundamental understanding of this type of evidence which is becoming increasingly common.

The source of self-DNA in touch deposits recovered from forensic contexts may reasonably be shed corneocytes from hands, DNA residues in fragmented cells, shed nucleated cells from hands, transferred cells from elsewhere on the body, or cell-free DNA. We have seen that recoverable DNA is lowered substantially by washing hands or handling items before sampling, but it then builds back up over time, at a rate that tends to vary between individuals [115, 39]. Whether this is due to removing “sheddable” cells from hands, removing transferred cells or DNA from elsewhere or removing sweat containing cell-free DNA is currently unclear. However, DNA is recovered from washed hands after wearing gloves, suggesting that hand-endogenous DNA of some origin is a viable

source [12]. Further exploration of relative contributions of hand-endogenous and -exogenous DNA contributions to touch deposits is warranted, along with more specific localization of DNA sources inside and outside a cellular infrastructure. This knowledge would allow better understanding of the actual mechanism of deposition and transfer of DNA, which is currently ambiguous and known only to be highly variable.

Determination of the origin of DNA in touch deposits can guide the design of preservation, recovery and analysis methodology, perhaps by improving techniques to target DNA by protein association, fragment length or cellular infrastructure. The study of cfDNA in forensics is relatively recent and optimizing the recovery and analysis protocol would improve results of touch deposit testing and characterization of cfDNA efforts. A more comprehensive picture of where the DNA in "touch DNA" comes from will provide valuable information to practitioners seeking to utilize touch DNA typing results in a courtroom context, and will contribute to the ongoing effort to understand and predict the circumstances of DNA transfer in a forensic context more reliably.

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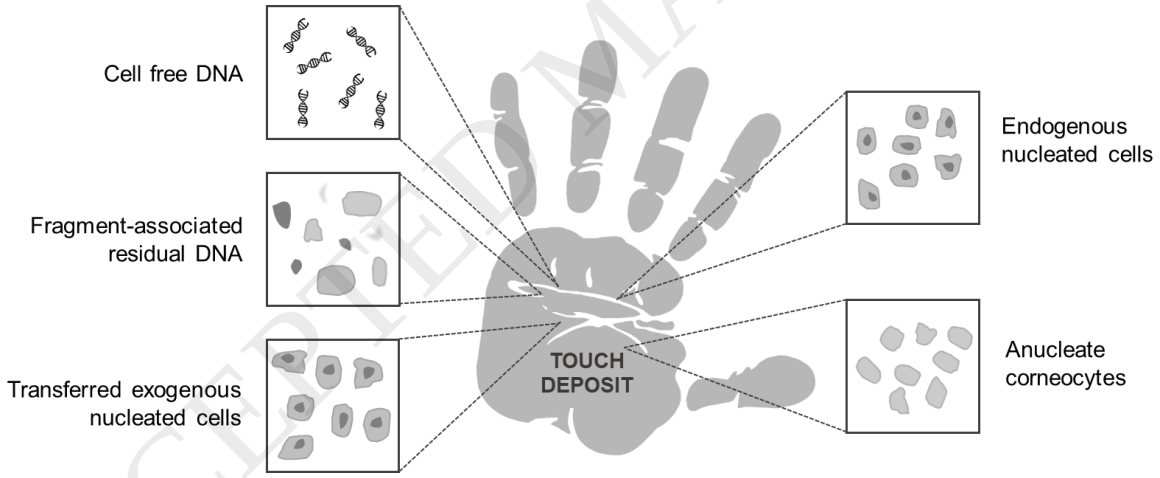

Figure 1. Concept map of potential sources of DNA deposited by touch/handling. It is currently well established that individuals may leave behind detectable DNA when they handle items, but the anatomical origin of that DNA remains unsolved. It is possible that the DNA typically recovered from handled items in forensic scenarios comes from nucleated cells from hands, anucleate cells from hands, nucleated cells transferred onto hands from elsewhere, residual cell fragments (including free nuclei) from hands or from outside a cellular architecture in sweat on hands or residual transferred body fluids.

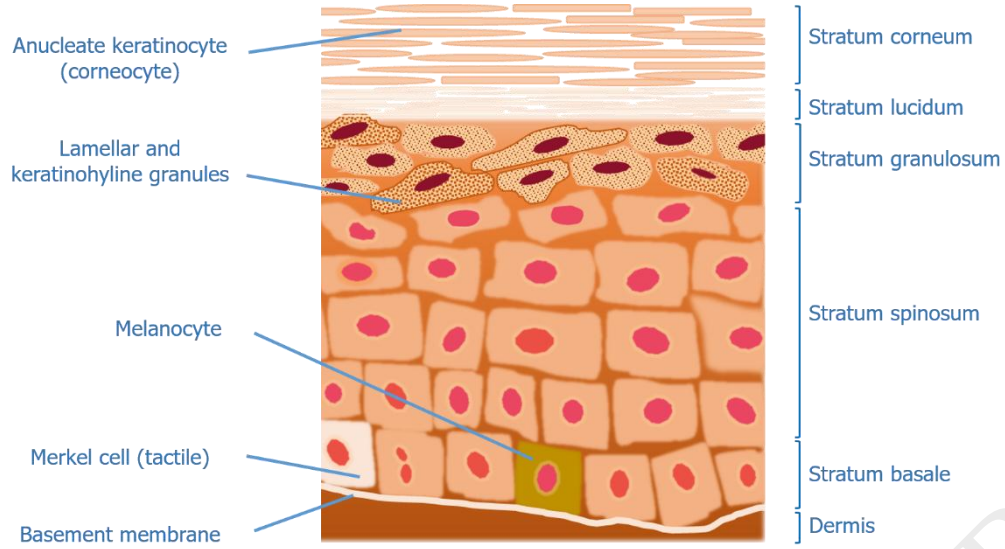

Figure 2. Epidermal layers in the thick skin of the palms of the hands distinguished by cell types. The process of terminal differentiation occurs as cells move up to the outermost layer of skin, generating an outer layer consisting of flattened keratinocytes lacking nuclei (corneocytes) made mostly of keratin filaments joined tightly together in a lipid- and protein-dense, highly cross-linked matrix [121].

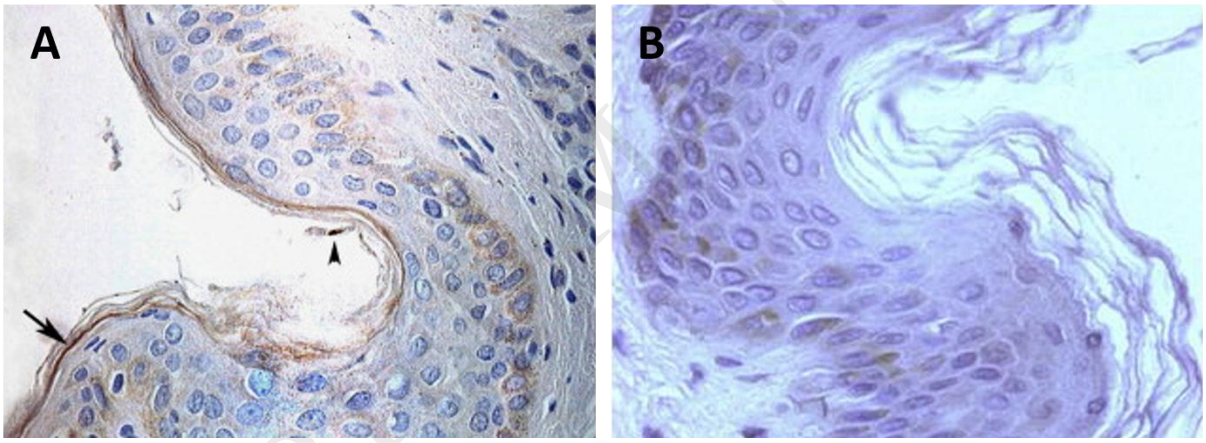

Figure 3. Images of outer epidermal layers processed and stained for ssDNA. (A) Outermost layers of human skin from neck of autopsy subject antibody stained for ssDNA. Published by Kita et al. [130]. Authors report "positive staining reactions were localized to the stripped nucleus like object (arrowhead) and the uppermost layer of stratified cornified cells (arrow)." (B) Outermost layers of human skin from autopsy subject. Published by Zoppis, et al. [115]. Authors detected no positive signal in either replicate sample, "demonstrating no fragmented nuclear DNA was present."

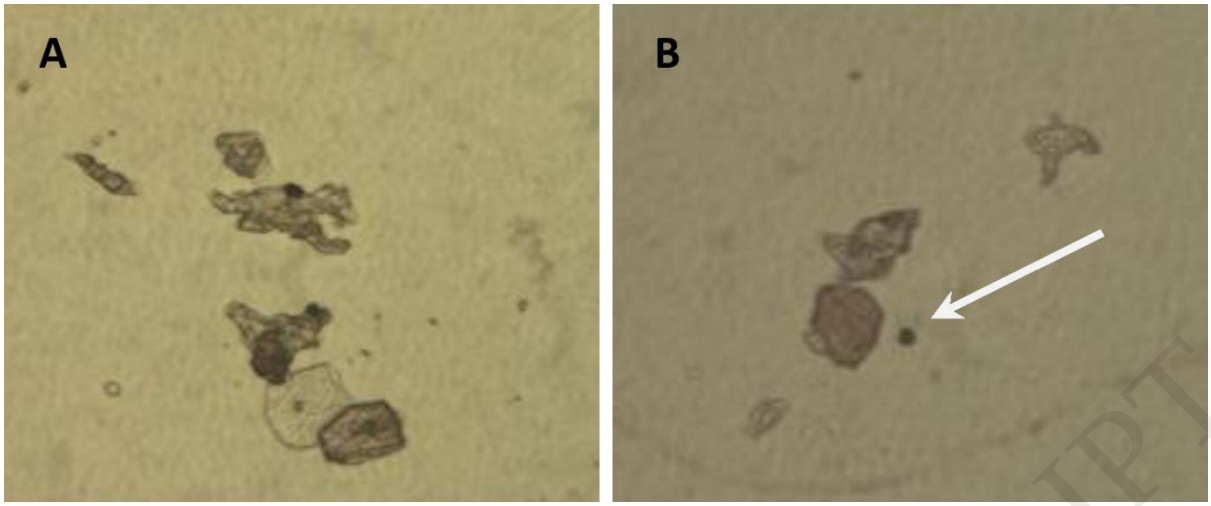

Figure 4. Images from fingerprints deposited 1 hr after washing without interim contact, stained with iron haematoxylin-anol solution. (A) Nucleated cells and anucleate corneocytes visible under direct light microscopic examination (× 400). (B) Anucleate corneocytes and "stripped nucleus" (indicated by arrow) visible under direct light microscopic examination (× 400). Published by Oleiwi, et al. [131].

Table 1: Summary of studies reporting published amounts of DNA recovered from various touched or handled items. Updated and adapted from table published by Meakin and Jamieson [33].

Surface	Length of contact	Nature of contact	Quantity recovered (ng)	Author	Publication Year
Swabbing of hand	-	-	2-150	van Oorschot and Jones	1997
plastic knife handle, mug, glass	15 min	holding	7-34	van Oorschot and Jones	1997
Swabbing of hand	-	-	0.1-6.4	Bright and Petricevic	2004
new lower bed sheet	1 night	sleeping	0-8	Petricevic et al.	2006
glass slides	5 sec	pressure	0-2	Allen et al. [35]	2007
paper	30 sec	pressure	0-110	Sewell et al.	2008
door frame	1 min	grabbing	0->0.2	Raymond et al. [36]	2008
cartridge casing	30 sec	handling	0.3-0.7	Horsman-Hall et al.	2009
cotton	10-15 sec	rubbing	6-12	Goray et al.	2010
plastic	10-15 sec	rubbing	0.4-0.5	Goray et al.	2010
melamine-coated board	10 sec	pressure	0-160	Kamphausen et al.	2012
glass	1 min	holding	0-5	Daly et al.	2012
fabric	1 min	holding	0-15	Daly et al.	2012
wood	1 min	holding	0-169	Daly et al.	2012
infant's clothing	1 min	rubbing	0.3-9	Goray et al.	2012
plastic block	1 min	rubbing	0-2.5	Goray et al.	2012
plastic syringe	10 sec	holding	0-80	Poetsch et al.	2013
glass slides	brief	fingerprint pressure	0-17.6	Thomasma and Foran	2013
knife handle	1 min x 4	simulated regular use	~ 1-10	Meakin et al. [37]	2015
glass slides	15 sec	fingerprint pressure	0-1.5	Oleiwi et al.	2015
glass	10 sec	pressure	0-5	Goray et al.	2016
knife handle	brief	grip/stabbing	0-4.8	Samie et al.	2016
non-porous cables	brief	fingerprint pressure	0-3	Lim et al.	2016
plastic tubes	10 sec	holding	0.04-3.8	Fonnelop et al.	2017
car steering wheel	2-60 min	holding	0.21-134	Kirgiz and Calloway	2017
plastic cable ties	brief	used to bind objects	0-39.8	Steensma et al. [38]	2017
polycarbonate board	brief	fingerprint pressure	0-3.5	Tobias et al.	2017

Table 2: Summary of selected published studies of trace DNA recovered under scenarios including varying levels and patterns of transfer and re-deposition.

Activity/Scenario	DNA Results	Comments	Author	Publication Year
A and B hold hands, B touches tube	Usually mixture, B as major; more partial profiles with time between contacts; A as single source on tube only if immediate transfer and A is "good shedder" while B is "bad."	Depends on individuals A and B "shedding", increased PCR cycles used	Lowe et al.	2002
A speaking, no direct contact with floor	A's profile detected up to 69 cm after sitting/kneeling, 115 cm after standing.	Detection closer to subject after 30 seconds or less	Port et al.	2005
A and B shake hands, B touches glass beaker; A touches beaker, then B touches beaker.	Usually second or only person to touch object was major contributor, exceptions for "better shedders."		Farmen et al.	2008
Purses/wallets used by A, "stolen" by B and kept for varying times	0.9-28.1 ng on owned items, 30% had non-owner alleles; 3.1-15.4 ng transferred to clean wallet by "robbery;" 90% wallets used for 1-2 weeks pre-"robbery" yielded mixtures.	"Robber" profile resolvable in 40% wallets; non-owner DNA may not be "robber"	Raymond et al. [100]	2009
3 specific mock case transfer scenarios tested	0-9.2 ng; transfer % varied by case	More variables present than could be accounted for in transfer prediction	Goray et al.	2012
3 people in filmed 20min social interactions over drinks	mixtures of 2-12 contributors found on most items: glasses, table, chairs, jug, hands; usually primary or most recent handler was major but not always	DNA often but not always transferred by touching or item to item contact, sometimes "bi-directional transfer"	Goray and van Oorschot	2013 and 2015
A grips clean tube, A shakes B's hand then grips tube, B shakes C's hand then A shakes B's hand then grips tube	Complete A profile, partial B seen on tubes	Foreign DNA from 1 hr before experiment seen; probable partial C profile seen at similar levels	Davies et al.	2015
A and B shake hands for 2 min, B touches knife	0-5 ng; Both A and B as major profile, more often B.	Mostly mixtures, a few single source, some foreign DNA	Cale et al.	2016
Subject "stabs" with knife after regular daily activity	0.05-4.8 ng; 3% stabber as single source, 80% stabber as major, 12% unresolvable mixture possibly including stabber, 5% inconclusive.	Knife handles usually display stabber + foreign DNA; major resolvable at some not all loci, minor not from co-workers	Samie et al.	2016
A deposits handprint, stabs with knife, then new handprints. B, C, D repeat actions with the same knife.	All participants' alleles present on knife, 3rd handler often largest contributor	Foreign DNA in all initial handprints, non-self DNA picked up from knife + self DNA decreases with more handprints	Buckingham et al.	2016
Male/Female engage in talking, touching face/hands then male simulates urination.	Low levels female DNA found on outside/waistband of male's underwear, also on penile swab, not on inside underwear.	Profiles not found if 6 hr delay after urination.	Jones et al.	2016
Cloth rubbed on A's neck, then on B's hand, B's hand rubbed on bag/cloth	0-103 ng; 22% cloth had A's complete profile, 40% partial; 70% B's hand had A's profile.	Better transfer rates with cloth as final item than with plastic, if B wears gloves, then B profile seen less but not 0.	Helmus et al.	2016
A and B have 10 min conversation side by side without touching.	No detectable evidence of speaking partner DNA on other partner's T-shirt		Fonneløp et al.	2017
A "attacks" B by grabbing/ pulling from behind, 20 sec struggle	A's profile seen in 76% of B T-shirt samples, exceptions when A is "low shedder;" limited B DNA on A's shirt.	No correlation between shedder status and when B detected on A's shirt.	Fonneløp et al.	2017
A uses knife regularly, then shakes B's hand and stabs with the knife	1-10 ng from "regular use;" A and B alleles seen after "stabbing," and 1 week later; B seen as minor only.	Foreign DNA in 1-person regularly used knives, possibly domestic partner	Meakin et al.	2017